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Epitope Distribution of Randomly Established Monoclonal Antibodies against Human Type II DNA Topoisomerases[1]

Ayako Sakaguchi,[*,2,3] Mitsuko Miyaike,[†,2] Kazufumi Kuroda,[†,2] Naohito Nozaki,[‡] Masato Tanaka,[§] Makoto Hibino,[*] Yasuo Fujii,[*] Satoshi Kato,[‖] and Akihiko Kikuchi[*,4]

[*]Nagoya University Graduate School & Faculty of Medicine, 466-8550; [†]Mitsubishi Kagaku Institute of Life Sciences, 194-8511; [‡]Kanagawa Dental College, 238-8580; [§]Tokyo Denki University, 350-0394; and [‖]Institute of Development, Aging and Cancer, Tohoku University, 980-8575

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The epitopes of about 100 monoclonal antibodies against human type II DNA topoisomerase were mapped along the enzyme molecules. Although they were randomly and independently established, epitope sites were unevenly distributed the toward N-terminal or C-terminal region. We suggest that the central catalytic domain is hidden inside the molecule and inaccessible to the antigen recognition sites. Using antibodies, we demonstrate the distinct localization of isoforms of Topo II in cultured cells. Some particularly useful antibodies are listed.

Key words: DNA topoisomerase, epitopes, immunoblotting analysis, indirect immunofluorescent staining of cultured cell, monoclonal antibodies.

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DNA topoisomerases are classified into two categories: type I topoisomerases, which transiently cut one of the two strands resulting in one linking change in every step of the reaction, and type II topoisomerases, which act by creating a transient double strand break, thus affecting two linking changes in one step. In most cells, both type I and type II enzymes are present. ATP requiring type II topoisomerase was first recognized due to its particular ability to catenate and decatenate or to unknot entangled DNA rings. At the end of DNA replication, two daughter molecules are thought to intertwine with each other. Type II topoisomerase is essential for separating the daughter DNA strands because this reaction cannot be carried out by type I topoisomerase (*1*).

The structure of type II topoisomerase is highly conserved from bacteria to human beings. Eucaryotic type II topoisomerase consists of an ATPase domain, a breakage/rejoining domains, and a putative regulatory domain, fused to form a single molecule (*2*). Lower eucaryotes have only one type II topoisomerase, but higher eucaryotes such as mammals and birds (and probably frogs and fish) have two genetically distinct isoforms of type II topoisomerase designated Topo IIα and Topo IIβ (*3–5*). The *in vitro* activities of Topo IIα and Topo IIβ are almost equivalent, although a knockout experiment suggested that their functions must differ. A Topo IIα-deficient homozygous egg scarcely divides (Sekimizu, K. and Akimitsu, N., unpublished observations), while Topo IIβ knockout mouse embryos grow but die at about the time of birth (*6*).

Both Topo IIα and Topo IIβ are nuclear enzymes, but their precise localization in the nucleus and profiles of cell cycle–dependent expression differ considerably (*7, 8*). In short, during cell cycle progression, the level of Topo IIα reaches a maximum in G2/M phase. Rapidly proliferating tumor cells maintain a high expression level at interphase, and Topo IIα can be used as a pathological marker for tumor diagnosis (*9*). Topo IIα localizes in the metaphase chromosome and is thought to be one of the major components of the chromosome scaffold (*10*). On the other hand, Topo IIβ is constantly expressed throughout the cell cycle and is distributed diffusedly in the nucleoplasm. Yet, there are some controversial observations as to whether Topo IIβ accumulates in the nucleolus, heterochromatin region, or some other distinct regions in the nucleus (*11*).

As we believed that good antibodies might be useful for distinguishing the two isoforms in the cell cycle and studying the difference of their precise localizations in the nucleus, we isolated as many as monoclonal antibodies against Topo IIα and Topo IIβ as possible. We particularly concentrated on antibodies against Topo IIβ, since its exact function and localization are unsolved. Here we classified our randomly selected monoclonal antibodies according to their epitopes and discuss some structural aspects of type II topoisomerases.

MATERIALS AND METHODS

Preparation of Monoclonal Antibodies—The cDNA of human Topo IIα (kindly provided by Dr. J. Wang, Harvard University) was expressed in *Escherichia coli* under the control of a T7 promoter (*12*). In a preliminary trial, the protein was purified by SDS-PAGE and bands correspond-

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[2] These persons equally and independently contributed to this work.
[3] Her work was supported in part by The Ito Scholarship Foundation, Japan.
[4] To whom correspondence should be addressed at: Laboratory of Medical Mycology, Institute for Disease Mechanism and Control, Nagoya University Graduate School & Faculty of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550. Tel: +81-52-744-2458, Fax: +81-52-744-2459, E-mail: aki@med.nagoya-u.ac.jp
Abbreviations: Topo II, topoisomerase II; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; DAPI, 4,6-diamidino-2-phenylindole.

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ing to 170 kDa were cut out, minced, mixed with Freund's adjuvant, and injected subcutaneously four times every two weeks, into Balb/c mice. The splenocytes were fused with myeloma cell lines SP2/0 and P3-X63Ag8, 653 and the culture media were screened by enzyme-linked immuno absorbent assay (ELISA) using purified *E. coli* expressed human Topo IIα. Among 157 positive clones, only 7 recognized human Topo IIα by ELISA using purified human Topo IIα and also by immunoblotting analysis of crude human cell lysates. The remaining 150 clones could produce antibodies against *E. coli*–produced human Topo II and recognize it, but not the native antigen. Thus we changed our protocol, omitting the selection step using the *E. coli*–produced antigen, but selected directly with a partially purified human Topo II preparation. Also, we modified our protocol in such a way that without the purification step by gel electrophoresis, the antigen was prepared solely as an inclusion body of induced *E. coli* lysate, because the major bands appearing in SDS-gel electrophoresis cross-reacted with anti-Topo II antibody (Fig. 2A).

For the preparation of monoclonal antibodies against Topo IIβ, Topo IIβ cDNA [kindly provided by Dr. I. Hickson, Oxford University (*5*)] was expressed in the same way and the inclusion body was used as an antigen for injection into mice. We also used two synthetic oligopeptides, C1 and C2, with the unique C terminal sequence region of human Topo

TABLE I. **List of synthetic peptides.** Peptides C1 and C2 were used as antigens (Fig. 6). Peptides T2F, T2M, and T2P and peptide T5/459 were used as competitors for the T2 and T5 regions, respectively (Fig. 2C).

Name	Peptide Sequence
C1	SDFPTEPPSLPRTGRARKE
C2	KKTTTPKGKGRGAKKRK
T2F	NNLISIWNNGKGIPV
T2M	VEHKVEKMYVPALIFGQLL
T2P	TSSNYDDDEKKVTGGRNRYRAKLC
T5/459	TEGDSAKTLAVSGLGVVGRDKYGVFPLRGK

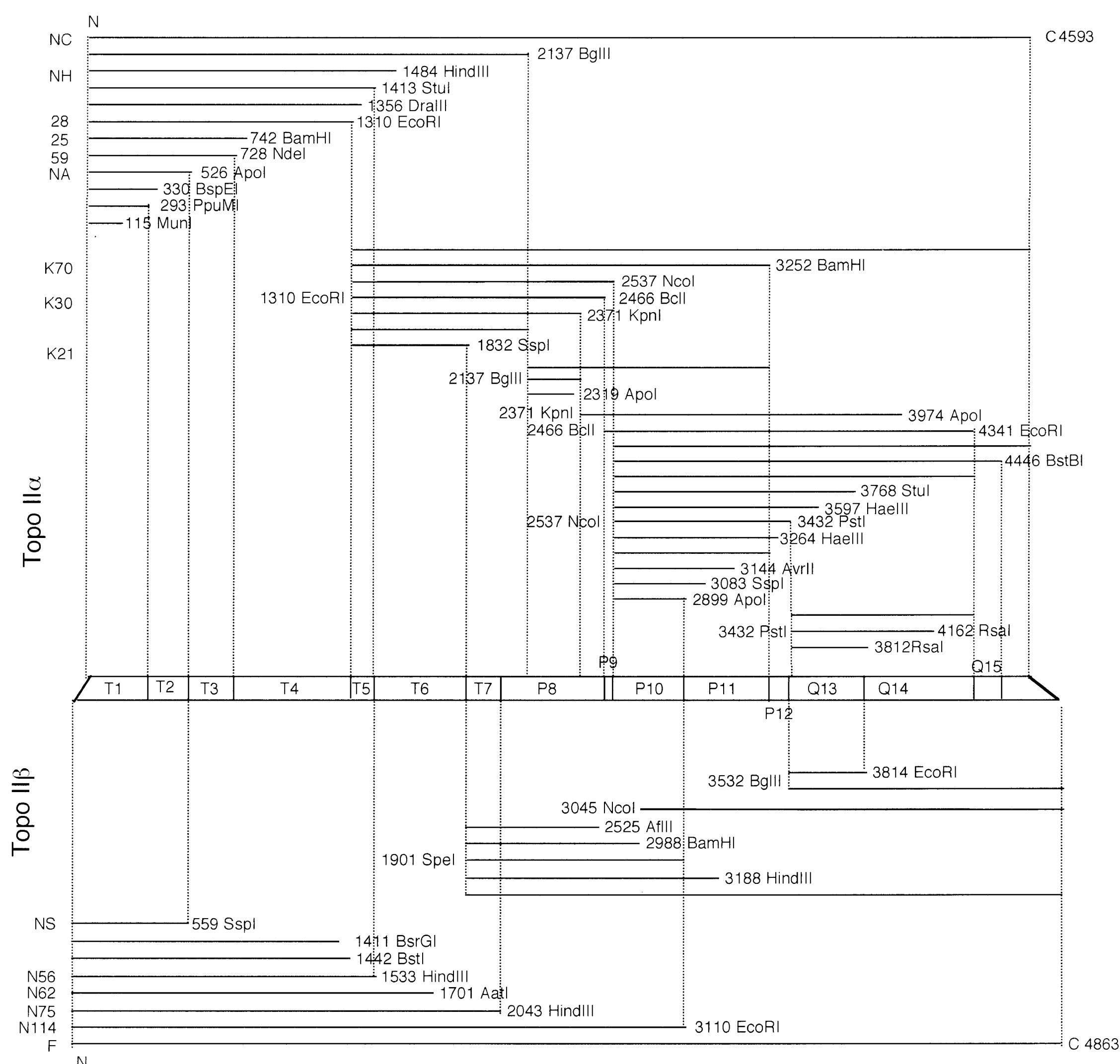

Fig. 1. **Diagram of cloned fragments in the T7 expression vector system.** Each horizontal line represents part of the cDNA of human Topo II cloned in T7 vector. The start and end of each fragment are indicated by the nucleotide number from the N terminal ATG, followed by the restriction site. The name of each clone appearing in the text is given to the left of the line. The bar in the center is divided into 16 regions (see in the text); the lines above it represent Topo IIα clones, and those below, Topo IIβ clones.

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IIβ as an antigen (Table I). Other synthetic oligopeptides with the specific N terminal region sequences of Topo IIα and Topo IIβ, and also the C terminal sequence of Topo IIα failed. In some cases, we used a partially purified native Topo II (mixtures of α and β isoform) from the human cultured cells as an antigen.

Construction of Plasmids Expressing Human Topo II Fragments—To determine epitope location, we first divided the Topo II molecule into three parts, T, the N terminal one-third, corresponding to the Gyr B/Par E subunit of *E. coli* type II topoisomerase, P, the central portion, corresponding to the Gyr A/Par C subunit, and Q, the C terminal one-third. The boundary between T and P is separated by a *Hpa*I site at 1,992 bp (from the start ATG) in Topo IIα and at 2,040 bp in Topo IIβ. The boundary between P and Q is slightly different in Topo IIα (*Pst*I site at 3,432 bp) and Topo IIβ (*Bgl*II site at 3,532 bp). Each of the three regions was subdivided arbitrarily as T1–T7, P8–P12, Q13–Q15, where appropriate restriction sites were available. Each fragment, as shown by the bars in Fig. 1, was cloned in the T7 expression vector, and transformed into an *E. coli* BL21 (DE3) host (*12*). Cells were grown in 1.5 ml of LB-Amp medium and proteins were induced by the addition of 1 mM IPTG, followed by a further 4 h of culture with vigorous shaking. Crude lysates were prepared by adding 50 μl of 2× sample buffer of SDS-PAGE to each cell pellet, heating at 100°C for 3min, with storage at –20°C. 1–0.2 μl of crude lysate was used for immunoblotting analysis.

Immunoblotting Analysis—The protein bands separated by SDS polyacrylamide gel electrophoresis were blotted onto nitrocellulose membrane filters in transfer buffer containing 50 mM Tris base, 380 mM Glycine, 0.1% SDS, and 20% methanol in a dry blotting apparatus at 150 mA for 90 min. Membranes were briefly blocked with 10% skim milk in TBS (10 mM Tris pH 8.0, 0.15 M NaCl) followed by incubation with each monoclonal antibody (1/100 dilution in TBS) at 4°C over night. The membranes were then washed extensively with TBT (0.1% Tween 80 in TBS) and incubated with Horse Radish Peroxidase conjugated antibody (1/3,000 dilution in TBS) for 2 h at room temperature. After extensive washing with TBT, followed by a brief rinse with water, the membranes were stained with a Konica immunostaining HPR-1000 kit for the detection of peroxidase using 4-chloro-1-naphtol.

Construction of Plasmids Expressing Topo IIβ Fragments in Yeast Cells—Plasmids were constructed to express YFP (yellow fluorescent protein)-Topo IIβ fusion proteins using the yeast expression vector pADHY01 (Fig. 3) (*11*). The regions corresponding to nucleotides 3799–4836 (β-7), 3958–4836 (β-8), 4144–4836 (β-9), 4318–4836 (β-10), and 4489-4836 (β-11) of the mouse Topo IIβ cDNA were amplified by PCR (*13*). The 5′-primers were 7F (β-7), 8F (β-8), 9F (β-9), 10F (β-10), and 11F (β-11), and the 3′-primer was 1R. All the primers used for the construction of the fusion genes are listed in Table II. The amplified products were digested with *Bsr*GI and *Xma*I and inserted into the vector using the multicloning site of pADHY01.

TABLE II. **List of mouse Topo IIβ primers.** These primers were used to construct plasmids expressing Topo IIβ fragments in yeast cells (Fig. 3).

5′-Primers	Sequence
7F	5′-AATTGTACAAAGAATTTAGTGGAACACCAG-3′
8F	5′-AGATGTACAATCCTTGGTCAGATGATGAG-3′
9F	5′-AAGTGTACAAAGTTAAAGCATCTCCCATAAC-3′
10F	5′-ATTTGTACAAGTCAGAAGATGATTCAGC-3′
11F	5′-CTATGTACAAGAGAGCCCCTAAACAGAA-3′
3′-Primer	
1R	5′-CTCCCCGGGCACTTAATTAAACATTG-3′

Immunoblotting Using Yeast Proteins—All recombinant plasmids were transformed into haploid yeast strain CB018-A (MAT**a** *pep4::HIS3 prb1::hisG prc1::hisG ura3-1 trp1-1 leu2-3,112 can1-100*). Yeast transformation was carried out by Frozen-EZ Yeast Transformation II (ZYMO RESEARCH) and Trp+ transformants were obtained at 28°C. Cells were grown in 5 ml of SD (0.67% Yeast Nitrogen Base without amino acids, 2% glucose) supplemented with appropriate amino acids and 0.004% adenine at 28°C overnight. Whole cell lysates were prepared by disrupting the cells with glass beads (*13*). Proteins were separated by 10% SDS-PAGE, transferred to a nitrocellulose filter and analyzed by immunoblotting. The immobilized proteins were allowed to react with monoclonal antibodies against Topo II at a dilution of 1:10–1:50, incubated with an alkaline phosphatase conjugated secondary antibody, and detected by an alkaline phosphatase detection method (Promega). Expression of the hybrid proteins was confirmed using Anti-GFP (BOEHRINGER MANNHEIM) at a dilution of 1:1,000 (data not shown).

Indirect Immunofluorescence—HeLa cells were grown in DMEM + 10% FBS (GIBCO BRL) and plated on 8 well slide glasses (CEL-LINE/ERIE SCIENTIFIC) one day before fixation. All steps were carried out at room temperature unless noted otherwise. Samples were washed three times in phosphate-buffered saline (PBS) after each step. Cells were fixed in freshly prepared 4% paraformaldehyde in PBS for 20 min and permeabilized in 0.1% Triton X-100 in PBS for 5 min. Samples were incubated sequentially for 1 h each with PBS-diluted primary antibodies, 3G3, 3B6, 1A5, 2G11, 7G10, and 3H10, at a dilution of 1:20 and secondary antibody, rhodamine-conjugated goat anti-mouse IgG (CHEMICON), at a dilution of 1:500 at 37°C, and mounted in Vectashield with DAPI (Vector Labs). Samples were examined with an epifluorescence microscope (Olympus BX-60), and rhodamine and DAPI fluorescences were detected with U-MWIG and U-MWU filters respectively. Images were acquired with an ORCA-ER CCD camera (HAMAMATSU) equipped with IP Lab software (Scananalytics).

RESULTS AND DISCUSSION

Many Monoclonal Antibodies against Topo II—Monoclonal antibodies are of use, especially if one has a series of antibodies that recognize different epitopes along a large antigen molecule composed of several functional domains. Type II DNA topoisomerase is such a protein that consists of three functional parts: T, a eucaryotic counterpart for subunit B of *E. coli* DNA gyrase, including the ATPase domain; P, that of subunit A with cutting and rejoining domains; and Q, which plays some regulatory roles *in vivo* rather than possessing a catalytic function. We and several other groups have attempted to prepare Topo II–specific antibodies and, especially, antibodies that can distinguish between Topo IIα and Topo IIβ (*14*). As we had difficulty purifying large amounts of pure Topo IIα or Topo IIβ suffi-

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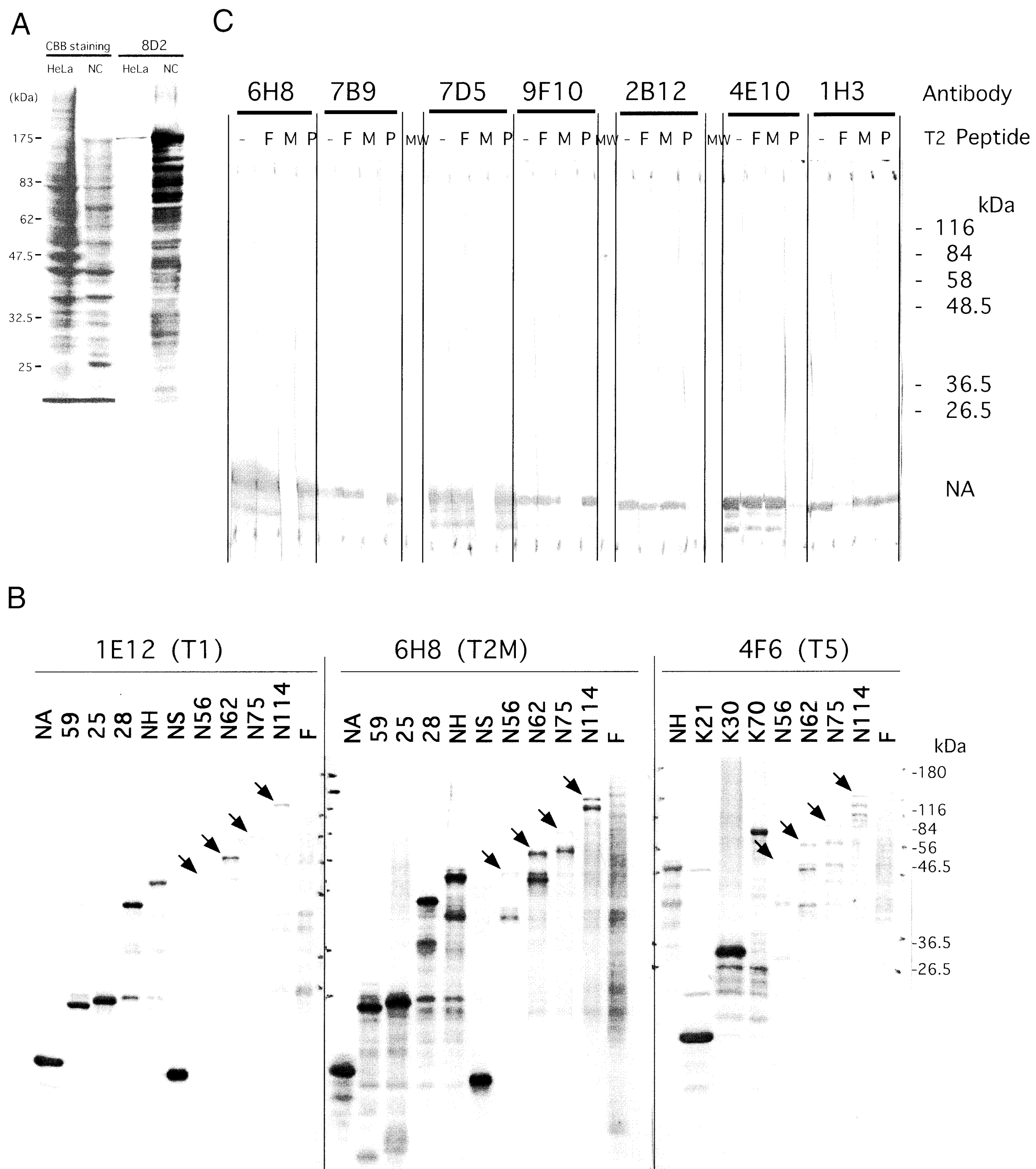

Fig. 2. **Immunoblotting analysis of Topo II fragments expressed by the T7 vector system.** (A) HeLa extract and *E. coli* extract (NC; see Fig. 1) containing the entire human Topo IIα were separated by SDS-PAGE. The left half of the gel was stained with Coomassie Brilliant Blue and the other half was subjected to immunoblotting analysis using the 8D2 antibody. (B) Several *E. coli* extracts were fractionated by SDS-PAGE followed by immunoblotting analysis using three antibodies with different epitopes. Note that antibody 1E12, which has an epitope near the N terminal end, recognized only the largest fragment, while the antibody with an epitope in an internal part (6H8, 4F6) recognized several smaller fragments. Particularly, the largest fragments made by Topo IIβ in lanes N56, N62, N75, N114, and F were relatively faint compared with the 2nd largest fragments (see the central and right panels), which were not recognized by the 1E12 antibody. Arrows indicate products from the 1st ATG codon. (C) Immunoblotting of fragment NA by various antibodies against the T2 region was performed in the presence of oligopeptides F, M or P (Table II). When the epitope was located in peptide M, the signal disappears, while other peptides have no effect on immunoblotting analysis. For example, the epitopes of 6H8, 7B9, 7D5, 9F10 are clearly located in the sequence of peptide M, while those of 2B12, 4E10 are in peptide P, and that of 1H3 is in peptide F.

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cient for use as antigens, we, instead, expressed the cDNAs of Topo IIα and Topo IIβ in *E. coli* under the control of the T7 phage promoter. As shown in Fig. 2A (NC), we could obtain a sizable 170 kDa protein band in SDS–polyacrylamide gels. Preliminarily we cut out this band and used it as an antigen in mice as described in "MATERIALS AND METHODS," and obtained one monoclonal antibody (3H8). Most of the clones recognized *E. coli*–produced human Topo II proteins but not human Topo II itself. Thus, we used a partially purified native Topo II from cultured cells for ELISA assay, because we needed antibodies that could recognize the native antigen.

We used four antigens in separated experiments and successfully obtained monoclonal antibodies: (i) *E. coli* lysates with an inclusion body of human Topo IIα; (ii) *E. coli* lysates with an inclusion body of human Topo IIβ; (iii) Oligopeptides of the C terminal region of human Topo IIβ (C1 and C2); and (iv) Partially purified human Topo II from cultured cells (mixture of Topo IIα and Topo IIβ).

Except in the 3rd case using oligopeptides as the antigen, we randomly isolated many monoclonal antibodies, and, later, determined the epitope region of each antibody by immunoblotting analysis using *E. coli*–expressed Topo II fragments. For example, as shown in a typical immunoblotting in Fig. 2B, antibody 1E12 and 6H8 recognized both fragments NA and NS. Thus their epitopes are in T1-T2 region and are common in Topo IIα and Topo IIβ. It can be noticed that a series of smaller bands appeared in addition to the expected fragment in immunoblotting analysis (Fig. 2, A and B). Those fragments were caused by premature termination and inappropriate initiation events. By immunoblotting analysis, antibody 1E12, which has an epitope close to the N terminus of the fragment, reacted only with the largest bands, while antibody 6H8 reacted with several smaller bands. Antibody 4F6 has an epitope in the T5 region, while fragments K21, K30, and K70 (start at the T5 region) mainly recognized the largest bands. On the other hand, several smaller fragments were recognized in lanes NH, N56, N62, N75, N114, and F (start from the upstream initiation point). For 1E12, we also used a smaller fragment (up to the *Mun*I site 115 bp from ATG) to localize in T1 region. Several antibodies, including 4A12, and 4E12 re-

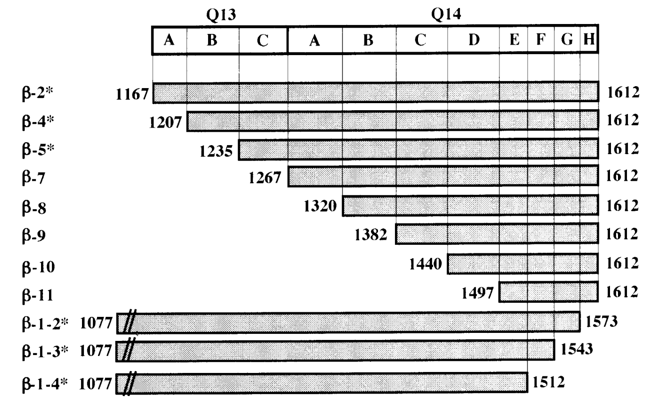

Fig. 3. **Q13 and Q14 were divided into three (A–C) and eight (A–H) pieces, respectively.** The plasmids constructed to express Topo IIβ fragments using the yeast expression system are shown below the schematic diagram of the Q13–Q14 region of Topo IIβ. Each construct carries a deleted form of mouse Topo IIβ fused to the C terminal end of YFP. The black box indicates the amino acid sequence of mouse Topo IIβ. The start and end of each fragment are indicated by the amino acid numbers from the N terminal end. Asterisks show constructs prepared in the previous work (*11*).

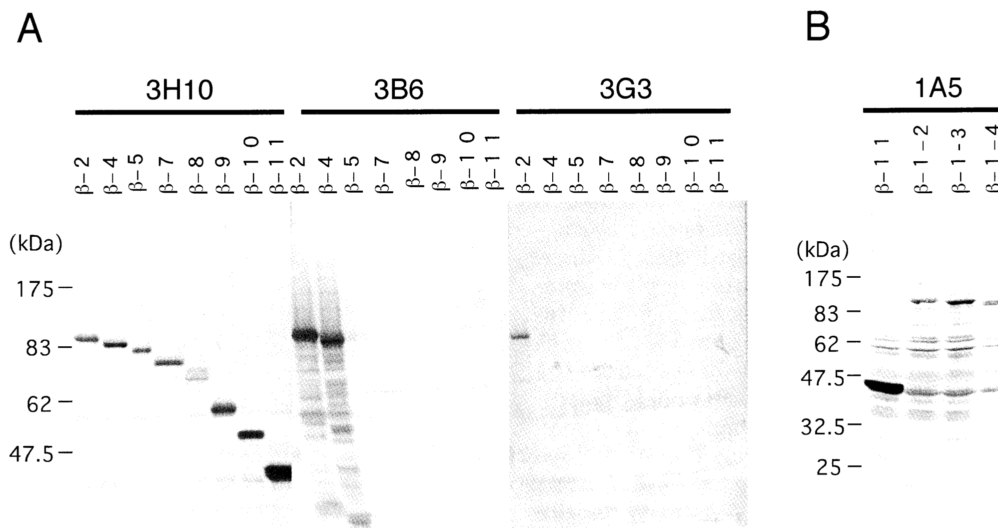

Fig. 4. **Immunoblotting analysis of Topo IIβ fragments including different parts of the Q13–Q14 region.** (A) The eight plasmids (β-2, β-4, β-5, β-7, β-8, β-9, β-10, and β-11) were introduced into yeast cells, and whole yeast lysates were fractionated by SDS-PAGE, followed by immunoblotting analysis using about 40 monoclonal antibodies raised against *E. coli*–expressed Topo IIβ. Since antibody 3H10 was raised against the peptide fragment named C2 (see Fig. 6), the signal of all bands appears (left panel). As 3B6 recognized the β-2 and β-4 fragments, we categorized its epitope into the Q13 B region (center panel) (see Figs. 3 and 6). The epitope of 3G3 was categorized into Q13 A, as well (right panel). (B) We further performed immunoblotting analysis of β-1-2, β-1-3, and β-1-4 fragments using seven antibodies (1A5, 2G11B5, 4E9, 7G10, 8H4, 11A9, and 11G7) that recognized the β-11 fragment at (A) (Fig. 3). As shown in the panel, 1A5 recognized all fragments, so we categorized its epitope into Q14 E. All epitopes determined in this analysis are listed in Fig. 6.

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acted with this small peptide but 1E12 failed to recognize it (data not shown). The further localization of the epitope for 6H8 is described below.

Further Subdivision of Epitope Loci Using Synthetic Oligopeptides—Since there were many monoclonal antibodies whose epitopes were located in the T2 region, such as, 6H8, 7B9, 7D5, 9F10, 2B12, 4E10, 1H3 *etc.*, we prepared several oligopeptides (Table I) to test the competition in immunoblotting analysis. If an epitope is included in the oligopeptide, positive signals in the immunoblotting must be cancelled. As shown in Fig. 2C, positive bands shown by 6H8, 7B9, 7D5 and 9F10 were cancelled by peptide M, the 2B12 and 4E10 bands disappeared with peptide P, and the 1H3 band was cancelled by peptide F, so that the T2 region was further subdivided into three parts. Peptide T5/459 was used as a competitor for the T5 region and the presence of this peptide did not cancel the immunoblotting reaction of 5F8, 1D1, 4F6, 7D12, 8F2, or 8H11. These antibodies recognize as few as 20 peptides in the T5 region (data not shown).

Why is the Distribution of Epitope Loci Uneven?—Surprisingly, the distribution of epitope loci is uneven. They are primarily restricted to two regions, T1, T2, and the Q region as summarized in Fig. 6. Why did we not obtain any monoclonal antibodies with epitopes in the T6~P11 region (the central and catalytic regions)? One reason might be that these regions do not contain suitable epitopes since this part of the human Topo II sequence is highly similar to that of mouse. This explanation is refuted by the fact that we obtained numerous antibodies against several oligopeptides with common catalytic domains as well as the Topo II fragment made in *E. coli*, all of which recognized *E. coli*–produced Topo II fragments, but showed little cross-reaction with native whole Topo IIα or Topo IIβ molecules by ELISA and by immunoblotting analysis, as described in "MATERIALS AND METHODS." Thus we argue that the antigenic sites of these regions might be folded inside in native Topo II molecules and, therefore, antibodies against these loci could not gain access to the epitope and were discarded during the ELISA selection step. This explanations is supported the yeast Topo II structure determined by X-ray crystallographic analysis (*15*).

The reason there were no β-specific antibodies in the N-terminal region is also puzzling, since there is an α-specific sequence in the T1 region. When we reexamined *E. coli*–expressed Topo IIβ fragments, we realized that the fragments starting from the 1st ATG codon were very few (compared with the intensity of the largest fragments as indicated by arrows in N56, N62, N15, N114 of Topo IIβ with those of 28, NH of Topo IIα in Fig 2B). Judging from the intensity of bands by 1E12, more fragments might be produced beginning from an internal methionine codon beyond the T3 region (as the third fragment from the top of Topo IIβ appears in lane N56–N114 of Fig. 2B, panel 4F6), where a specific sequence for Topo IIβ is hard to find. To obtain a Topo IIβ–specific antibody with an epitope at the N terminal region, it might be necessary to prepare larger amounts of pure native Topo IIβ, free from Topo IIα. Thus, in this study, the epitopes of all the Topo IIβ–specific monoclonal antibodies were located in the Q regions.

The Similar Biased Distribution of Epitopes Was Seen in Q13–Q14—As reported previously, we constructed a series of plasmids expressing different parts of the Q13–Q14 region of Topo IIβ (*11*). In addition, we constructed five plasmids, β-7, β-8, β-9, β-10, and β-11, and divided the Q13–Q14 region into three (A–C) and eight (A–H) subregions respectively (Fig. 3). We have picked up about 40 of monoclonal antibodies raised against *E. coli*–expressed Topo IIβ proteins and determined their epitope regions in detail using these constructs (Fig. 4). As shown in Fig. 6, these epitopes clustered only in Q13 and Q14 E, F, with none mapping in the Q14 A–D region. It might also be the case that the Q14 A–D region is folded to the inside of the Topo II molecule where antibodies have little access. Unfortunately, there are no X-ray crystallographic data for the Q region.

We have reported that mammalian Topo IIβ is sequestered in a subnuclear compartment of the yeast nucleus and that two regions (amino acids 1207–1234 and 1513–1573) are essential for this specific localization (*11*). These

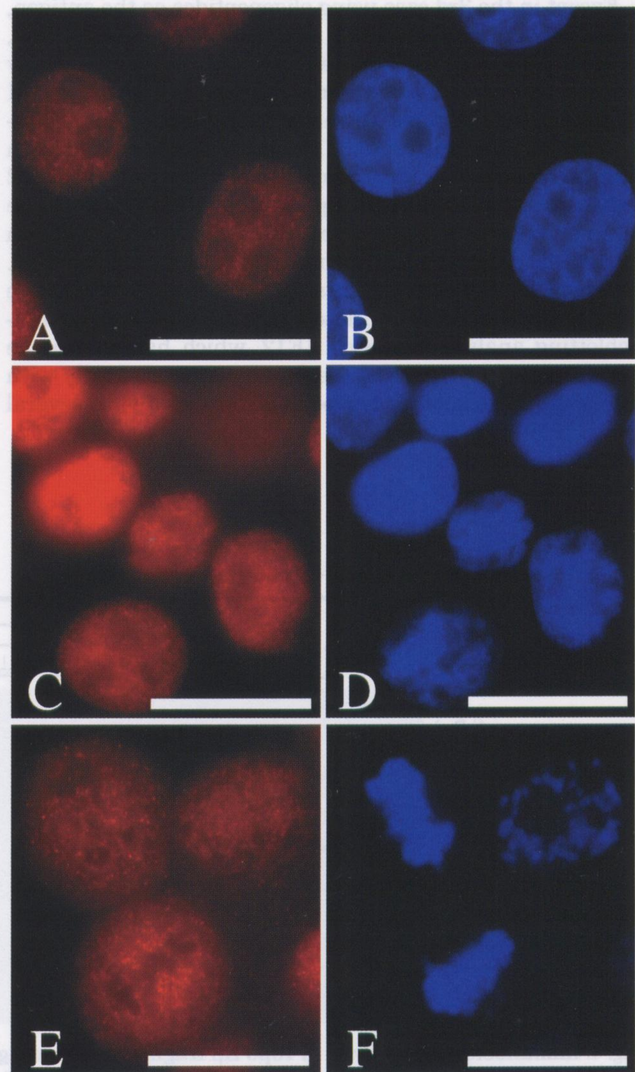

Fig. 5. **Immunofluorescence staining of interphase (A, B, C, and D) and mitotic (E and F) HeLa cells.** Cells were labeled with β-specific monoclonal antibodies 3B6 (A), 3G3 (C), and 1A5 (E), and counterstained with DAPI (B, D, and F). The labeling patterns with 2G11, 7G10, and 3H10 were indistinguishable and not shown here. The bar indicates 10 μm.

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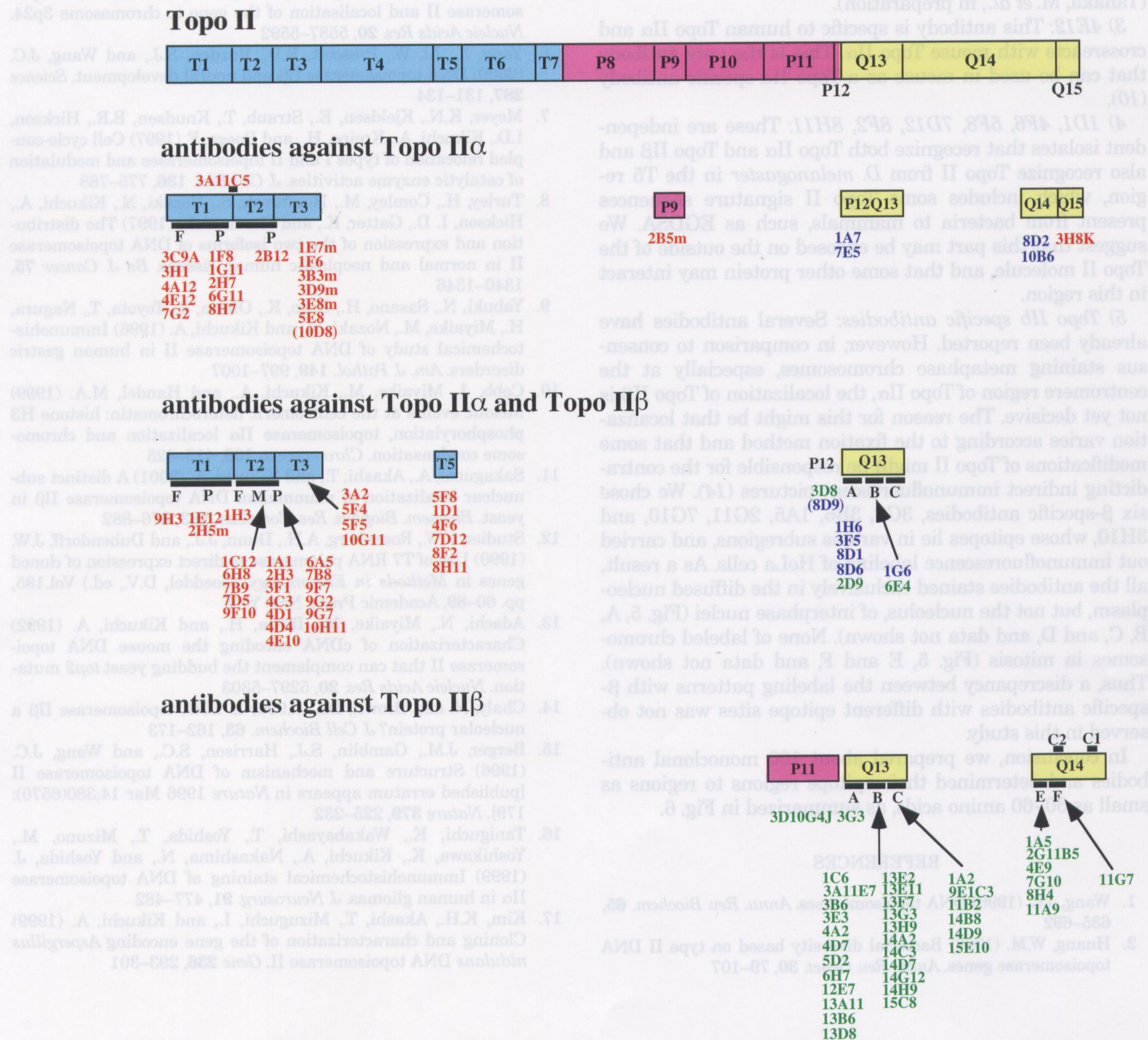
putative subnuclear localization signals reside in the Q13 B and Q14 F, G subregions. These regions must be exposed to the outside and can be recognized by proteins as localization signals. This hypothesis is in good agreement with the above results of antibody accessibility.

Some antibodies whose epitopes were located at the C terminal region (Q14 E, F), could scarcely recognize a fragment carrying the Q14 A–D region. This suggests that some epitope regions might be unexposed in the presence of the Q14 A–D regions. There may be a strong interaction between these two regions. This might also be another reason that few antibodies were obtained against the Q14 A–D region.

Notes on Some Useful Antibodies—1) 8D2: This human Topo IIα-specific antibody with an epitope in the Q14 region has been studied extensively as a diagnostic marker



anti-peptide antibodies

C2				C1
1A11	1F10	2D7	4E2	3A12
1B2	1G5	2F1	4G4	3C2
1C8	1H3	2G11	5A7	4B4
1C11	2B3	2H2	5C6	5B8
1D4	2B4	3A3	5E8	6G4
1D8	2B9	3B7		9A11
1F1	2B10	3H10		9E1F9
				10F6

Fig. 6. **List of epitope locations.** Each antibody is first classified according to its specificity for Topo IIα (the first row) or Topo IIβ (the third row); many antibodies recognized equally both Topo IIα and Topo IIβ (the second row). Then the antibodies are arranged in the region T1 to Q15. The antibodies in red were raised using *E. coli*–expressed human Topo IIα cDNA; those in green were raised using *E. coli*–expressed human Topo IIβ cDNA as an antigen. Those in blue were raised with partially purified Topo IIα and Topo IIβ from cultured cells. Those in black and listed separately are monoclonal antibodies against each peptide. Subdivisions F, M, and P were determined by immunoblotting assays in the presence of competing oligopeptides, while A, B, C, and E, F were designed as shown in Fig. 3.

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for tumor cells (*9*). Also, it is reported that a high level of 8D2-positive cells after therapeutic treatment indicates a poor prognosis (*16*).

2) 6H8, 7B9, 7D5: These three antibodies recognized both isoforms of Topo IIα and Topo IIβ and their epitopes are in the T2M region. However each has a different specificity, namely, 6H8 equally recognizes Topo II from frog, fly, filamentous fungi (*17*) and yeast, while the other two are relatively specific to vertebrates and not to flies or yeast. These antibodies block Topo IIα and Topo IIβ activity *in vivo* when injected into cell cultures, and arrest cell division (Tanaka, M. *et al.*, in preparation).

3) 4E12: This antibody is specific to human Topo IIα and crossreacts with mouse Topo IIα. This is the only antibody that can be used in mouse as a Topo IIα-specific antibody (*10*).

4) 1D1, 4F6, 5F8, 7D12, 8F2, 8H11: These are independent isolates that recognize both Topo IIα and Topo IIβ and also recognize Topo II from *D. melanogaster* in the T5 region, which includes some Topo II signature sequences present from bacteria to mammals, such as EGDSA. We suggest that this part may be exposed on the outside of the Topo II molecule, and that some other protein may interact in this region.

5) Topo IIb specific antibodies: Several antibodies have already been reported. However, in comparison to consensus staining metaphase chromosomes, especially at the centromere region of Topo IIα, the localization of Topo IIβ is not yet decisive. The reason for this might be that localization varies according to the fixation method and that some modifications of Topo II might be responsible for the contradicting indirect immunofluorescent pictures (*14*). We chose six β-specific antibodies, 3G3, 3B6, 1A5, 2G11, 7G10, and 3H10, whose epitopes lie in various subregions, and carried out immunofluorescence labeling of HeLa cells. As a result, all the antibodies stained exclusively in the diffused nucleoplasm, but not the nucleolus, of interphase nuclei (Fig. 5, A, B, C, and D, and data not shown). None of labeled chromosomes in mitosis (Fig. 5, E and F, and data not shown). Thus, a discrepancy between the labeling patterns with β-specific antibodies with different epitope sites was not observed in this study.

In conclusion, we prepared about 100 monoclonal antibodies and determined their epitope regions to regions as small as 30~60 amino acids, as summarized in Fig. 6.

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